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Chapter

Heterochromatin Dynamics in Response to Environmental Stress in Amazonian Fish

Daniele Aparecida Matoso, Maelin da Silva, Hallana Cristina Menezes da Silva, Eliana Feldberg and Roberto Ferreira Artoni

Abstract

Transcriptionally inactive portions of genomic DNA, condensed with histones and architectural proteins, are known as heterochromatic regions, often positive C band. The advent of epigenetics and new methodological approaches, showed that these regions are extremely dynamic and responsive to different types of environmental stress. The relationship of the constitutive heterochromatin with the transposable elements inactivation, especially from the Rex family, seems to be a frequent condition in fish. In this manuscript we review the existing knowledge of the nature and function of these genomic regions, based on species-based studies, with a focus on species of fish from the Amazon region.

Keywords: environmental stressors, transposable elements, adaptive response, *Colossoma*, *Hypancistrus*

1. Introduction

The genomic DNA of eukaryotic organisms combines with histone proteins to form chromatin. Chromatin is classified into two forms: euchromatin (de-condensed region, rich in genes and transcriptionally active) and heterochromatin (condensed, transcriptionally silent) [1–3]. This early classification was based on differing dye-responses and condensation profiles [4]. Heterochromatin, in turn, can be classified into constitutive heterochromatin and facultative heterochromatin, the former being preferably assembled in regions that house repetitive elements, such as satellite DNA and transposable elements [2, 5, 6]. The latter is preferentially assembled in genes related to the regulation of organismal development. The idea that the material is strongly related to the heterochromatinization of one of the X sex chromosomes in female mammals is known as the Lyon hypothesis [7].

Recent studies have shown that both constitutive and facultative heterochromatin are regulated dynamically and are responsive to various stressful stimuli. It is also known that while these changes in chromatin structure can potentially help organisms adapt to new environments, they can also produce aberrant phenotypes [8, 9] and diseases in humans [10, 11]. They are also related to the aging process [12]. In this review, we will discuss the dynamics of heterochromatin localization, obtained from different studies in fish from the Amazon region (**Figure 1**).

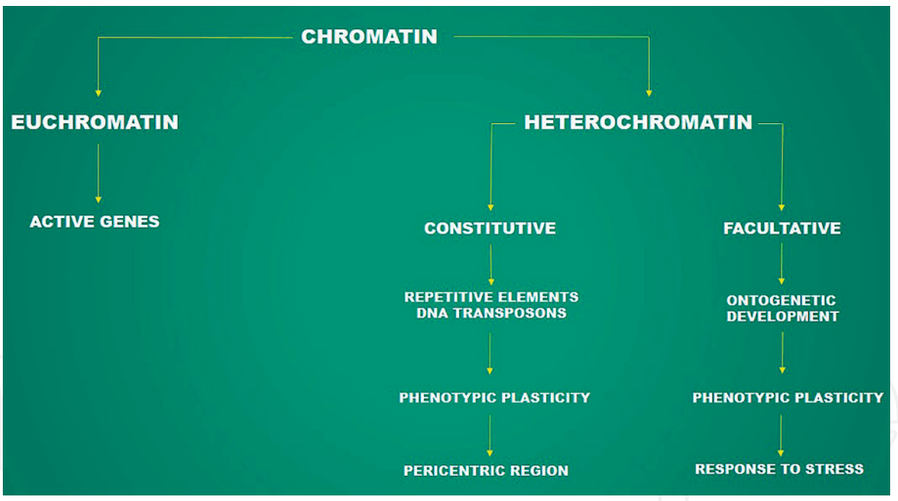

Figure 1.
General distribution of chromatin in eukaryotos. This schematization compiles information from several studies. Here, euchromatin is represented as the portion of chromatin with transcriptionally active genes. Meanwhile, heterochromatin can be divided into two groups depending on constitution and activity. The constitutive herochromatin is composed of repetitive sequences and transposable elements, distributed in pericentromeric and terminal region of chromosomes that occasionally participate in phenotypic plasticity, through the regulation of chromatin and dispersion of transposable elements, uneven crossing-over or rearrangements. On the other hand, the portion of facultative heterochromatin, regulated during ontogenetic development, is related to the response of environmental stress or laboratory simulated, allowing plasticity and environmental adaptation.

2. Molecular characteristics of heterochromatin

Facultative heterochromatin is traditionally considered to have a more plastic structure than constitutive heterochromatin.

At the molecular level, facultative heterochromatin is composed of transcriptionally silent chromatin regions, which condense or decondense, thus allowing transcription in temporal and spatial contexts [13]. Therefore, facultative heterochromatin formation appears to be directly linked to the different isoforms of histone H1. Here, under the facultative heterochromatin formation model proposed by [14], the different H1 isoforms can take on unique functions via specific changes in chromatin structure.

The concept of facultative heterochromatin was developed to explain the phenomenon of dose compensation in mammalian cells. The X chromosome, inactive in female mammalian cells, is subject to a monoallelic suppression of genes that depends on numerous chromatin modifiers, resulting in extensive condensation [15]. This process involves non-coding RNA (ncRNA) called X_{ist}, which is exclusively expressed by the inactive X chromosome. This ncRNA is responsible for post-translational modifications of histones, among which the most common forms include H4 lysine 20 methylation (H4K20me), H3 lysine 27 trimethylation (H3K27me3), and H3 lysine 9 methylation [16, 17]. Another important variation is the incorporation of the histone macro H2A, while Polycomb (PcG), Polycomb repressor complex 2 (PRC2), and PRC1 are also involved in the process. It is generally considered that Polycomb (PcG) proteins play a central role in the formation of facultative heterochromatin, with the most powerful histone modification being the methylation of H3 lysine 27 (H3K27me) [13].

Hypoacetylation of histone tails, binding of the HP1 protein with H3K9me2/3, and ubiquitination of H2A lysine 119, as well as the presence of histone macro H2A, appear to provide a molecular signature for the composition of facultative heterochromatin [13]. The fact that facultative heterochromatin is maintained across cell generations, with PcG proteins, ncRNA, and trans- acting transcription factors as

participants, shows that this form of heterochromatin may be largely responsible for phenotypic differences, which can be inherited or arise spontaneously in response to environmental challenges or ontogenetic development [13, 15].

Some studies have shown that the HP1 protein undergoes changes during the cell differentiation process [18]. Such changes are considered indicative of a highly conserved regulatory mechanism for the assembly of heterochromatin in response to environmental stress [12]. The phosphorylation function of HP1 is flexible, allowing responses to various stimuli and permitting more finessed cellular adaptation to environmental changes [12]. This suggests that epigenetic changes, mediated by heterochromatin, constitute a quick and efficient mechanism for generating flexible cell tolerance responses to environmental stress [12].

It has been established that the composition and formation of the constitutive heterochromatin are similar to that of the facultative heterochromatin regions, with these regions being hypoacetylated and containing histone H3 with hypermethylated lysine 9 (H3K9me) [19, 20]. The assembly of heterochromatic domains requires the joint action of a series of chromatin-modifying enzymes [12, 20].

In *Drosophila* and mammals, 12 factors appear to form the key components of constitutive heterochromatin in somatic cells. These include: histone H1 and its H2a/z variant, the chromatin-modifying enzymes SUV39h1, SUV39h2, SUV4-20 h1, SUV4-20 h2, Hdac2, HPα, and HP1β, the group of high mobility proteins such as Hmga1 and Hmga2, remodeling components such as Atrx, Trim28 co-repressor, and members of the Mbd protein family (methyl-binding domain) [21].

Cytologically, facultative and constitutive heterochromatin regions were indistinguishable. Therefore, cytogenomic studies that use conventional C banding to detect heterochromatin lack the resolution required to determine which type of heterochromatin is contained in each genomic region. However, this technique remains important for demonstrating the considerable genomic plasticity shown by organisms in the face of different environmental stimuli. It is beyond the scope of this review to conduct a detailed survey of the composition of both types of heterochromatin, as our aim is to demonstrate how changes in the process of heterochromatin modulation, via retroelement inactivation or gene expression regulation, affect phenotypic plasticity of Amazon region fish when confronting different environmental stressors.

2.1 Epigenetic adaptation and environmental stress: focus on selected Amazonian fish

The link between epigenetic adaptation and response to environmental stimuli is a topic that has been studied extensively in recent years. Several studies have reported the emergence of epigenetic plasticity and the consequent assembly of heterochromatin in various organisms when exposed to stress [22–25]. Such studies have shown a close relationship between gene silencing, via heterochromatin assembly or DNA methylation, and transcriptional regulation.

In Amazonian fish species, a large number of heterochromatic patterns have been reported following chromosome C banding treatments. These have been related to a wide variety of environmental stressors.

In the loricariid catfish *Hypancitrus debilittera*, Silva et al. [26] identified several polymorphisms, including nucleolar organizing regions (RONs), 5S, and 18S rDNA ribosomal sites, mainly via C banding patterns. Likewise, an intense morphological staining polymorphism, designated as P1, was found in this species. In addition, pronounced morphological variation in the body striping pattern (termed P2, P3, P4, and P5) was recorded, suggesting a correlation with the observed chromosomal polymorphism (**Figure 2**). It was proposed that this morphological polymorphism

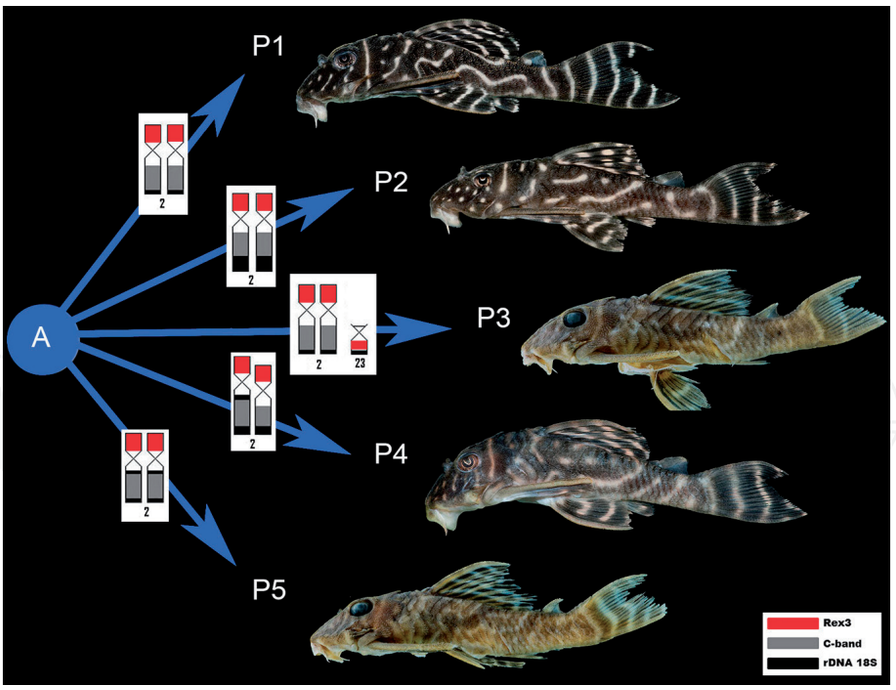

Figure 2.
Different morphological patterns (P1, P2, P3, P4, P5) and C-banding corresponding in Hypancistrus debillitera *highlighting the polymorphic pair 2, which presents a difference in heterochromatic block size and 18S rDNA sites. (more details see: [26]).*

was the result of a position effect variegation. Position effect variegation (PEV) was first described for the *Drosophila* gene *white,* and is a classic example of how constitutive heterochromatin operates, since, due to translocation events, this gene is variably silenced if located in a position adjacent to the pericentric heterochromatin [12]. The PEV phenomenon has been recorded widely in eukaryotes, from yeasts to humans [3, 21, 27], and can explain the observed variation in *Hypancitrus debilittera* body stripe patterns.

Heterochromatin-related PEV modifiers are called variegation suppressors [Su (var)], while those related to euchromatin are termed variegation enhancers [E (var)] [21]. Su (var) mutations weaken the formation and maintenance of heterochromatin, while E (var) mutations decrease euchromatin or allow heterochromatin expansion [5].

In *Hypancistrus debilittera,* C banding shows a variation in the distribution of heterochromatin in the five known different patterns (P1, P2, P3, P4, and P5), where heterochromatin assembly at gene regions, related to the staining pattern and consequent silencing, could result in the morphological polymorphism observed in the species. However, Silva et al. [26] reported no numerical or structural chromosomal rearrangements related to the emergence of polymorphic heterochromatin patterns. Therefore, to test the hypothesis of operative gene silencing by assembling PEV-like heterochromatin, the Rex3 transposable element was mapped and found to be present and associated with heterochromatic regions in multiple regions of the genome of this species [26]. This indicates that heterochromatin polymorphism may be associated with morphological polymorphism and other chromosomal polymorphisms found in the species (**Figure 2**).

Heterochromatin formation is strongly linked to transposable element inactivation [5]. Several studies have shown that depletion of Su (var) 3–9 (variegation-suppressing enzyme 3–9), which is a methyltransferase promoting H3K9 trimethylation [28, 29], can lead to the formation of mutant phenotypes, including abnormal chromosomal segregation, interruption of spermatogenesis (with links

to hypogonadism and infertility), and increased risk of tumorigenesis [16]. In *Drosophila,* this protein is essential for the maintenance of nucleolar stability, and its loss promotes nucleolar fragmentation. In addition, aberrant recombination of repeated DNA sequences results in rDNA locus instability [9]. This suggests that the heterochromatin, rDNA, and Ag-RONs polymorphisms seen in *H. debilittera* are directly or indirectly related to the functioning of such enzymes as Suvar3–9 since the polymorphisms of rDNA and Ag-RONs can be indicative of loss or gain of protein functions active in the assembly of heterochromatin.

Hypancistrus debilittera (family Loricariidae) is endemic to the Xingu River [30], and preferentially inhabits rapids [31]. Structural chromosomal polymorphisms are common in the Loricariidae, with karyotype plasticity marked by Robertsonian rearrangements [26, 32–34]. Therefore, the polymorphisms found in this species can be explained as either reflecting the cellular mechanisms of an adaptive response to the environment of the rapids or may simply result from the genomic plasticity of the family.

Identification of the molecular signature of heterochromatin, under assemblages in the genome of *H. debilittera* that block the harmful action of transposable elements such as Rex3, may lead to an understanding of the mechanism producing such a high degree of polymorphism in these organisms. However, it remains unclear whether the color polymorphism of *H. debilittera* is a PEV-like phenomenon, related to an adaptation to environmental stimuli and the assembly of heterochromatin correlated with the inactivation of retrotransposable elements (for example Rex3), or whether it is an efficient mechanism for increasing genetic variability in the face of environmental challenges (such as those found in rapids). Answers to these questions are likely to involve interrelation between the two possibilities.

In a conceptually linked study, Whitelaw and Martin [35] analyzed isogenic strains of mice and found morphological phenotypic variation related to the action of retrotransposons. According to these authors, the effects of the stochastic activity of retroelements on gene expression and the inactivation process of these elements indicate that somatic cells of individuals can be epigenetic mosaics, corresponding to the activity of each retrotransposon, and such activity can produce subtle phenotypic variations, even in genetically identical individuals.

In another study, using the corydoras catfish *Hoplosternum littorale* (family Callichthyidae) as a model, Silva et al. [36] found polymorphism related to the presence of multiple 18S rDNA sites, co-located with Rex3 retroelements, from individuals from polluted and unpolluted forest streams (*igarapés*) bordering the Amazonian city of Manaus. They reported Rex3 sites to be more conspicuous in samples from polluted environments than those from unpolluted environments. In addition, the C band showed heterochromatin polymorphism, which is common for species of the fish family Callichthyidae, with samples from polluted environments having more conspicuous blocks (Igarapé Mindú and Igarapé Quarenta). It has been suggested that this heterochromatin increase results either from a heterochromatinization process or from the addition of heterochromatin, caused by uneven crossing-over, duplication, or epigenetic mechanisms such as DNA methylation and chromatin remodeling. In a complementary study, Silva et al. [37] compared cytogenetically polluted and unpolluted aquatic environments of Manaus, using three species of Amazonian fish, *Pterygoplichthys pardalis*, *Semaprochilodus insignis,* and *Cichlasoma amazonarum*. The authors found an increase in Rex6 in *P. pardalis*, in Rex1, and Rex3 in *S. insignis* and in Rex6 in *C. amazonarum*. There was a correlation between Rex6 and constitutive heterochromatin and increased heterochromatin in *P. pardalis* individuals from a polluted environment. In this work, the authors suggest the retrotransposable element Rex 6 as a marker of inhospitable environments.

Examining the relationship between retroelements and heterochromatin polymorphism, Silva et al. [36], found a significant increase in heterochromatin in *Colossoma macropomum* exposed to cupric sulfate at 30% of the species LC_{50}, compared to the group control. Changes in heterochromatin levels were evident after 48 h of stress, and after 72 h, several chromosomes appeared marked with heterochromatin. In addition, there were significantly more copies of the Rex1 retroelement in individuals exposed to $CuSO_4$ for 72 h, when compared to the control. This element was mapped by FISH in animals exposed for 48 h to $CuSO_4$, and several chromosomes appeared stained.

A study of the parental species and hybrid offspring (commonly known as *tambacu*) of crosses between female tambaqui (*Colossoma macropomum*) and male pacu (*Piaractus mesopotamicus*), Ribeiro et al. [38] found variation in the heterochromatin pattern of hybrids as well as conspicuous patches of Rex3 and Rex6 retrotransposable elements. This result was interpreted as arising from the need for adjustments in cell division and introgressive hybridization, since the joining of two different genomes frequently leads to changes in the control of gene expression, DNA methylation, chromosomal rearrangements, and, consequently, transposable element mobilization.

Ferreira et al. [39] exposed *Colossoma macropomum* to three climate change scenarios of the Intergovernmental Panel on Climate Change (IPCC). They reported chromosomal heterochromatinization in individuals exposed to the A2 climate scenario, in which terminal and interstitial bands of bands were observed in several chromosomes. These correlated with the presence of Rex3 retrotransposon elements. In another study involving tambaqui, Costa et al. [40] detected different patterns in heterochromatin distribution associated with increased Rex3 expression, following exposure to the parasiticide Trichlorfon. The authors found that,

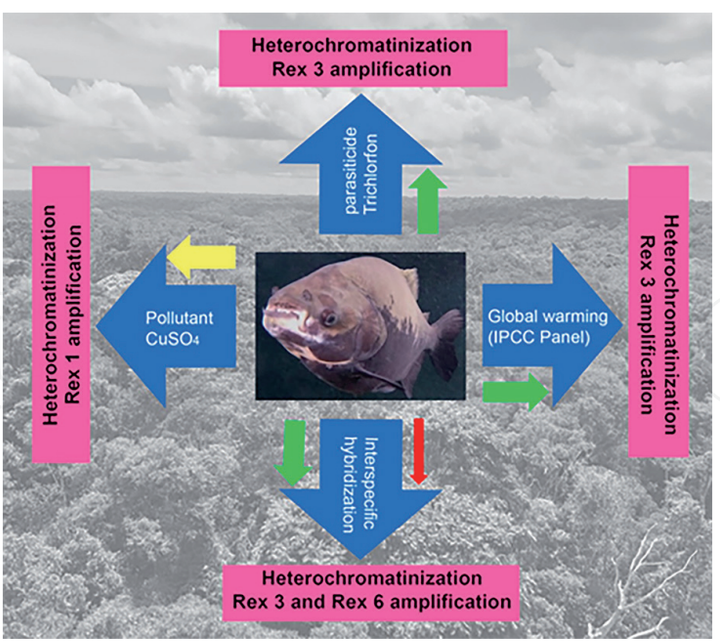

Figure 3.
The experimental data obtained in Colossoma macropomum *indicate that in all conditions of chemical stress, change in environmental temperature or shock between interspecific genomes, there was an increase in the amount of heterochromatin in the pericentromeric portion of all chromosomes of the complement or in the telomere portion, followed by an increase in the number of copies of the transposable elements. From left to right: Heterochromatinization and amplification of Rex1 in exposure to CuSO4 (yellow arrow); heterochromatinization and amplification of Rex3 in contact with antiparasitic Triclorfon and climate change provided for in the IPCC (green arrow); finally, amplification of Rex3 (green arrow) and Rex6 (red arrow) accompanied by heterochromatinization in a situation of interspecific artificial hybridization (more details see: [36–39]). Yellow arrow = Rex1, green arrow = Rex3 and red arrow = Rex6.*

after exposure to 50% of the established LC_{50}-96 h [41, 42] for the species, the organisms showed areas indicating active Rex3 elements as well as conspicuous heterochromatin marks in non-pericentromeric regions, with interstitial and terminal bi-telomeric markings. Such studies seem to show an intimate relationship between changes in the heterochromatic profile of *C. macropomum* and Rex3 retrotransposable element inactivation mechanisms.

The molecular composition of heterochromatin has not been elucidated in any of the aforementioned studies, although it has been correlated with Rex3 elements in *H. debilittera* [26] and *C. macropomum* [39, 40]. However, the role of retrotransposable elements in the regulation of gene activity seems undeniable. Mediated by heterochromatin, this produces widely variable phenotypes, which could help explain the enormous phenotypic plasticity observed in aquatic organisms in the Amazon. Future approaches focusing on methylation patterns in DNA and histone tails, in addition to detection of the molecular signature of heterochromatin assembled to inactivate both retroelements and other genes, should provide a clearer view of the adaptive processes developed by fish in the Amazon to deal with stressful environments.

In this context, tambaqui (*Colossoma macropomum*), a charismatic and often-studied Amazonian fish, is notable, as it shows the same responses to experimental simulation conditions, global warming conditions, treatment with antiparasiticides in cultivation, environmental pollution, and interspecific hybridization (**Figure 3**). It is not unreasonable to assume, under such circumstances, that the similarity of chromosomal behaviors under such a variety of stressors is casual and is likely to be the result of similar, if not identical, molecular and physiological mechanisms.

3. Conclusion

In conclusion, the species of Amazonian fish studied for heterochromatin assembly and retroelement dispersion (especially Rex 3) seem to respond dynamically and with remarkable similarity to a range of stressing stimuli.

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Conflict of interest

The authors declare no conflict of interest.

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